DETERMINATION OF GENES CONFERRING HOST SPECIFICITY IN GRAPE STRAINS OF *XYLELLA FASTIDIOSA* USING WHOLE-GENOMIC DNA MICROARRAYS

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ABSTRACT
Xylella fastidiosa (*Xf*) has many plant hosts and causes serious diseases of several crops and ornamentals. Strains of *Xf* can be classified by the hosts that may be infected. For example, grape strains do not infect oleander and the oleander strains do not infect grape. We are using a DNA Oligo-Microarray based on the genomic sequence of the *Xf* grape strain 'Temecula' as the reference strain for a genome-wide comparison with DNA from non-virulent strains. Our approach will determine genes unique to grape strains and thus presumably important in growth and virulence of *Xf* in grape. We hypothesized that the grape strain possesses several unique genes in comparison to other strains that do not infect grape. Initially 2526 of the 2574 predicted ORFs of *Xf* 'Temecula' were designed using the "pick70" software. We manually designed 70-mers oligos for 23 additional ORFs using the same criteria as the program. The remaining ORFs for which oligos were not designed had paralogs elsewhere in the genome with up to 100% identity. Test arrays have been made to determine optimal concentrations of spotted oligos (probes) using a subset of either four or eight probes. Optimal signal intensity was found for a probe concentration of 15-25 nM/ml. All eight probes tested hybridized with labeled DNA from both the *Xf* grape strain 'Temecula' and oleander strain 'Ann'. This indicated that the 8 hypothetical small genes used for the test array were conserved amongst these two genomes. Several quality control tests are underway before we use the full array. The full array includes 2551 70-mer oligos representing the full genome of the *Xf* grape strain 'Temecula'. These oligos were generated with a 5' amino linker that allows for covalent binding to aldehyde or epoxy coated slides, therefore minimizing the background.

INTRODUCTION
Some strains of *Xf* isolated from host plants other than grape do not sustain viable populations or are not virulent in grape. In particular, many of the almond strains of *Xf* do not infect grape (Almeida and Purcell 2003). Other studies provide evidence for host specificity among the *Xf* strains. On a whole genome level, grape strains of *Xf* were found to cluster together away from oak, plum, mulberry, and periwinkle strains using RFLP data (Chen et al. 1992, Chen et al. 1995). Pooler and Hartung (1995) divided the *Xf* in 5 groups (citrus, plum, grape-ragweed, almond, and mulberry) based on RAPD-PCR data. Most almond strains are genetically distinct from the grape strains but a few clustered within the grape-strain group whereas oleander, peach, and oak strains were distinct from other strains using RAPD-PCR, CHEF gel electrophoresis, and 16S-23S rRNA sequence analysis (Hendson et al. 2001). Reciprocal inoculation studies in the greenhouse showed that the OLS and PD strains of *Xf* were not pathogenic to citrus and that the ALS strain was not pathogenic to oleander (Feil et al. *unpublished*).

Based on previous analysis, we estimate that ~4% of the whole genome of the oleander strain is unique to that strain. We hypothesized that the grape strain also possesses ~4% of unique genes in comparison to other strains that do not infect grape. To identify these genes, we will use the grape strain 'Temecula' as a reference to perform pairwise comparison experiments via DNA hybridization using each *Xf* strain that is non-pathogenic to grape. By comparing a large number of strains that both colonize and cause symptoms in grape as well as strains that do not colonize grape we should be able to identify a relatively small number of unique genes that contribute to the virulence of grape by *Xf*.

OBJECTIVES
1. Identify host-specific virulence determinants of the *Xf* grape strain 'Temecula1a'.
2. Investigate the role of these specific genes in virulence.

RESULTS
Strains and Strategy of Screening
70-mer oligodeoxynucleotides were designed using 'ArrayOligoSelector' ('Pick70') software (http://arrayoligosel.sourceforge.net) based on the coding sequence of 2526 of the 2574 predicted ORFs of *Xf* 'Temecula1'. An additional 23 oligos were manually designed from the remaining unrepresented ORFs using the same criteria as 'Pick70', except that sequence 5' or 3' of ORFs smaller than 70 bases was added to obtain an oligo of the correct size. The remaining 25 ORFs are represented by paralogs with 100% identity found elsewhere in the genome. The designed oligos were generated with a 5' amino linker that has allowed for covalent binding to aldehyde or epoxy coated slides. The Final number of ORFs represented by gene-specific oligodeoxynucleotides on the arrays is 2551 not including negative and positive

controls. Recently we have optimized our hybridization process. A probe concentration between 15 – 25 nM/ml gave the highest signal following hybridization with labeled DNA. We have the oligos to print no fewer then 5,000 slides depending on the final concentration of the oligos and the number of slides printed during each printing. These slides represent the whole genome of a grape strain of *Xf* and we will compare this genome to the genome of about 15 other *Xf* strains non-pathogenic to grape as well as to at least 15 strains pathogenic to grape.

The host range of many strains of *Xf* has been studied and we will use this information in this study. We will use well-characterized strains of *Xf* that were found to not sustain viable populations in grape or to be non-pathogenic to grape. Some strains will be chosen based on their placement in phylogenetic trees after molecular analyses (i.e several almond, oleander, oak, peach strains, etc) These strains are listed in Table 1.

Table 1. Isolates of *Xf* that will be used in the study.

Name	Host	Origin	**Log CFU/g (±SE) in grapes**	Reference
Temecula	Grape	Riverside, CA	8.4 ± 0.1	Almeida et al. 2003
STL	Grape	Napa	8.3 ± 0.1	Almeida et al. 2003
Medeiros	Grape	Fresno	8.4 ± 0.1	Almeida et al. 2003
Dixon	Almond	Solano Co., CA	3.8 ± 0.1	Almeida et al. 2003
ALS7	Almond	San Joaquin, CA	4.5	Almeida et al. 2003
Manteca	Almond	San Joaquin, CA	3.9	Almeida et al. 2003
Ann1	Oleander	Riverside, CA	None	Almeida et al. 2003
Plum 2#4	Plum	Georgia	--	Hendson et al. 2001
Oak 88-9	Oak	Florida	--	Hendson et al. 2001
Oak 92-3	Oak	Florida	--	Hendson et al. 2001
OLS#2	Oak	Georgia	--	Hendson et al. 2001
5S2	Peach	Georgia	--	Hendson et al. 2001
5R1	Peach	Georgia	--	Hendson et al. 2001
4S3	Peach	Georgia	--	Hendson et al. 2001
ML1	Mulberry	Georgia	--	Chen et al. 1992
ML2	Mulberry	Georgia	--	Chen et al. 1992

Initial DNA hybridizations was done using microarray. The DNA microarray for the Temecula strain of *Xf* is now complete. We have purchased and spotted the oligonucleotides corresponding to each open reading frame of this strain on glass slides. We can readily produce as many DNA microarrays as we and other researchers will need. As noted above, the conditions for hybridization of DNA to this microarray has now been optimized. A probe concentration of 20 nM/µl gave the highest signal following hybridization with labeled DNA. We have collected all of the *Xf* strains noted in Table 1 that will be used in initial genome comparisons using the DNA microarray. We are in the process of extracting genomic DNA from these strains as well as many other grape strains of *Xf* and will hybridize to the DNA microarray very soon. The DNA is being sheared by sonication and being reciprocally labeled with Cy3 and Cy5 fluorescent dyes. Test hybridizations are being performed to enable us to determine threshold differences for use in genomic comparisons. Images of array spots were collected as 16 bit Tiff files by scanning washed slides using the GenePix 4000B laser Scanner (Axon Instruments, Union City, CA). The GenePix Pro 4.1 software program will be used for data collection to analyze the 16 bit Tiff files and for measuring signal intensities for each. The value for spot intensity will be normalized by subtracting the respective background intensity for each spot from the initial intensity.

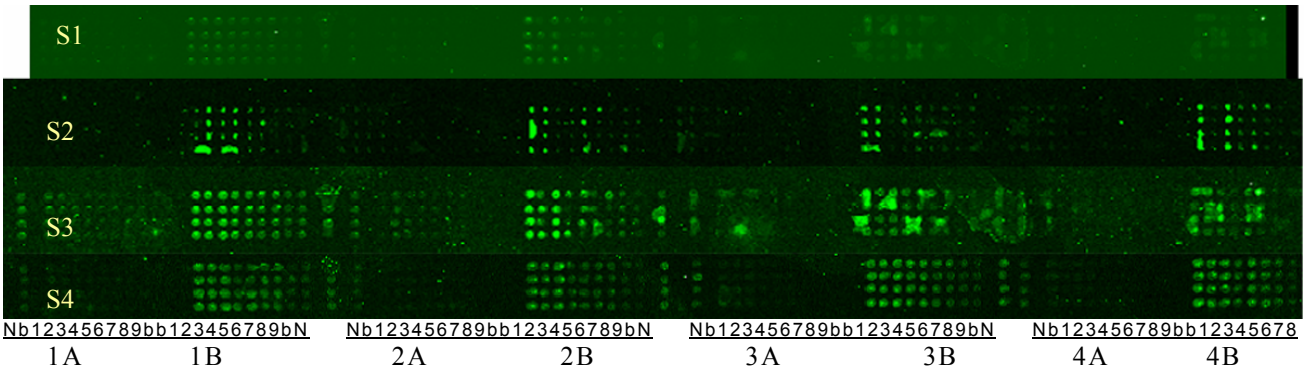

Figure 1. Combined images from four 70mer-oligo test arrays representing 8 ORFs. Each Slide (S1 – S4) was hybridized separately with cy3-labelled sheared DNA and a representative section of the resulting image was used for this figure. Oligos were spotted as in Table 1. N, negative control; b, buffer; 1, oligo concentration is 40 nM/ml; 2, 35 nM/ml; 3, 30 nM/ml; 4, 25 nM/ml; 5, 25 nM/ml; 6, 20 nM/ml; 6, 15 nM/ml; 7, 10 nM/ml; 8, 5 nM/ml; 9, 5 nM/ml. S1 and S2, epoxy-silane slides by Schott (Elmsford, NY; S3 and S4, by Telechem (ArrayIt™ Division, Sunnyvale CA). S1 and S3, hybridized with *Xf* 'Temecula' DNA; S2 and S4, hybridized with *Xf* 'Ann1' DNA.

Table 2: List of ORFs used in the Test Array in Fig 1.

Block	ORF	Function
1A	282	Hypothetical
1B	595	Hypothetical
2A	818	Hypothetical
2B	1812	Hypothetical
3A	2159	Hypothetical
3B	2255	Hypothetical
4A	2461	Hypothetical
4B	2696	Hypothetical

Upon completion of objective 1 putative grape-specific virulence genes will be identified for the mutagenicity experiment. To test the pathogenicity of the mutants, we will needle-inoculate grapes with the mutants and wild type *Xf* strains and check for pathogenicity. We will also examine the mutant cells (i.e. deficient in the unique genes to the grape strain) under scanning electron microscope (SEM) to determine their morphology in vitro and their behavior in planta. Future research to characterize virulence of these genes in various hosts has been proposed.

CONCLUSIONS

We have now completed the extensive process of identifying unique oligonucleotides suitable for use in the DNA microarray as well as determining the conditions for hybridization. The actual process of DNA-DNA hybridization on the oligonucleotide arrays should proceed quickly and we should soon have a list of genes unique to grape strains of *Xf*. Since we have already observed differences between strains of *Xylella fastidiosa* using amplified fragment length polymorphism (Feil et al, unpublished) and via cross-inoculation experiments we expect that such unique genes will be found and be predictive of host range and/or virulence. We expect that our analyses using this method comparing the grape strain to many other strains non-virulent to grape will provide a robust and complete set of unique genes to the grape strain of *Xf*. We have the oligos to print no fewer then 5,000 slides depending on the final concentration of the oligos and the number of slides printed during each printing. These slides represent the whole genome of *Xf* and should be invaluable to other scientists also interested in strain comparisons or gene expression analysis studies. The information gathered by this study can also be used to produce specific DNA markers for differential detection of *Xf* strains such as by PCR.

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